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THE CHRYSANTHEMUM RAY BLIGHT[1]

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(WITH FIFTEEN FIGURES)

The common name chosen for the disease at present under discussion is taken from the most conspicuous symptoms of the malady, a blighting of the corolla of the ray flower, resulting in poorly developed, discolored, one-sided heads (*fig. 1*).

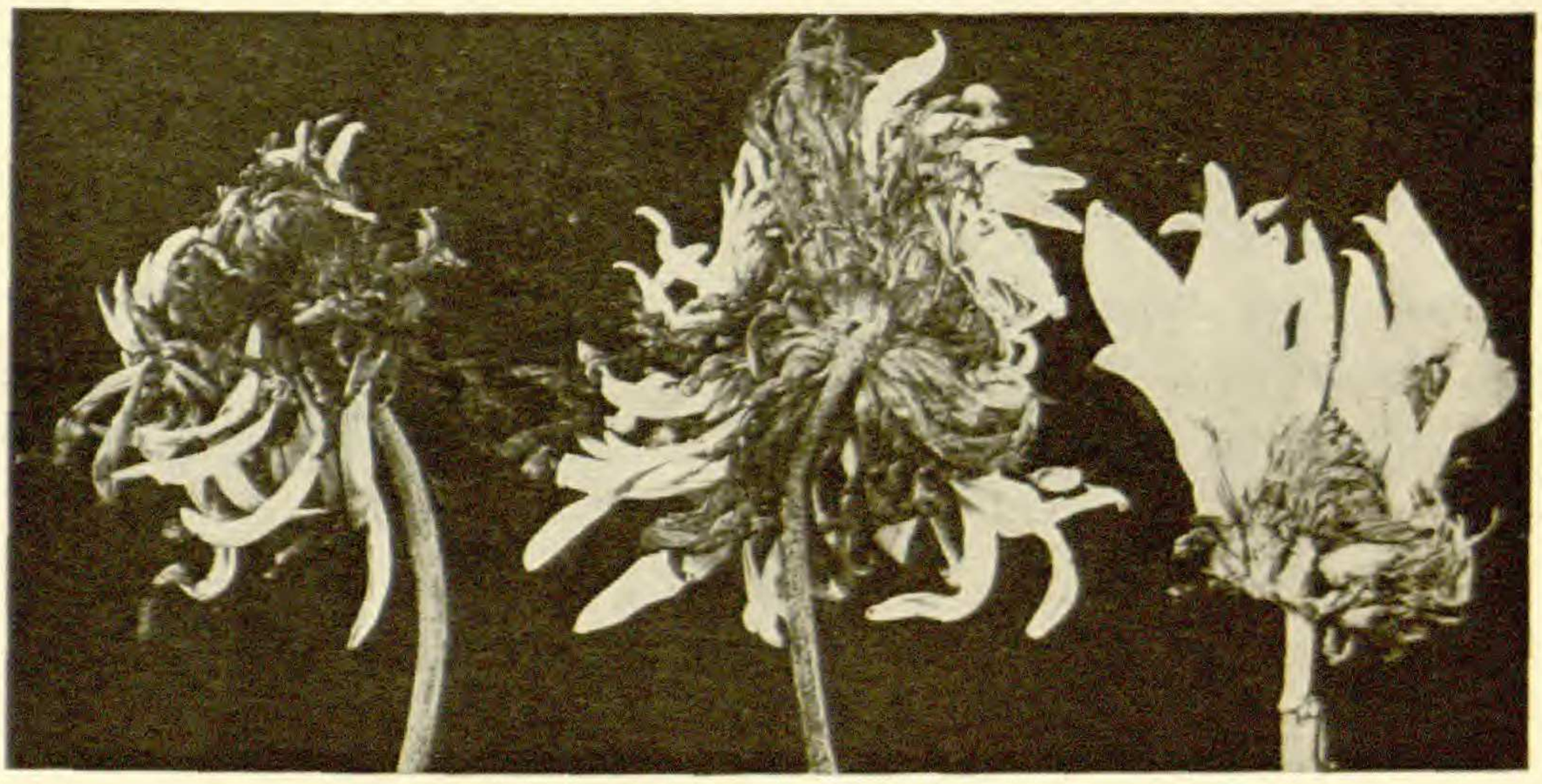

FIG. 1.—Three heads, showing disease as it naturally occurs.

Attention was first called to the disease by a letter from Fayetteville, N.C., dated November 13, 1906, and accompanied by specimens. The disease has been known at that place and has recurred for three years, each year with increasing destructiveness. It was first attrib-

[1] Contribution from the Laboratory of the North Carolina Agricultural Experiment Station, Raleigh, N. C.

uted by the owner of the plants to want of ventilation, but upon its recurrence the second year, the possibility of such a cause was precluded by the conditions. The disease was found upon plants in the open as well as upon those of the greenhouse. The first year it was found only upon late varieties, but this year it appeared upon all varieties in the owner's collection, the variety "Nellie Pockett" being particularly susceptible. The "Golden Wedding," a late yellow variety, always suffers badly in the collection in question. Since receiving the specimens from Fayetteville the disease has been found in Raleigh, and it may be of wide distribution.

SYMPTOMS

The disease is most conspicuous in the flower clusters, which it attacks usually upon one side, either in the bud or during various stages of blooming. The affected blossoms turn straw color or brownish, cease to develop, and wither, the discoloration proceeding from base toward tip on each individual flower, thus distinguishing the disease at once from many disorders which may resemble it superficially. If the case be severe, and a bud be attacked while still young, no rays will develop; the head will not open. If the attack be later, a portion of the head, one-half or two-thirds, or more or less, may develop normally and thus by contrast heighten the conspicuousness of the disease. Heads nearly open and apparently healthy may on the following day show one side far advanced in disease. The owner says, "it developed so fast that a large perfect bloom in the morning would be wilted by night."

The receptacle turns black, and the peduncle may likewise be blackened to a distance of one or two centimeters below the head. Later, shriveling and softening of the peduncle allow the head to nod in a characteristic manner.

On the affected plants the stems are often blackened for several centimeters, in a band more or less completely encircling the stem. Frequently this diseased region is associated with a leaf, the petiole of which also partakes of the discoloration.

MICROSCOPIC EXAMINATION

Under the microscope, diseased flowers, rays, corollas, receptacles, peduncles, and the diseased portions of the stem all reveal

the presence of a rather coarse, much branched mycelium that is easily recognized by its septa, which are numerous and stand out with an especially striking clearness (*fig. 2*). In the humid region between the flowers in the head, the mycelium becomes aerial, forming a loose floccose weft, visible to the naked eye. In the pith cavity of the blighted receptacle a similar profuse mycelial development occurs; while on old specimens, in culture dishes, pycnidia were also found identical with those which developed in the pure cultures to be described later (*fig. 3*). Only one fungus was seen and no bacteria whatever were visible.

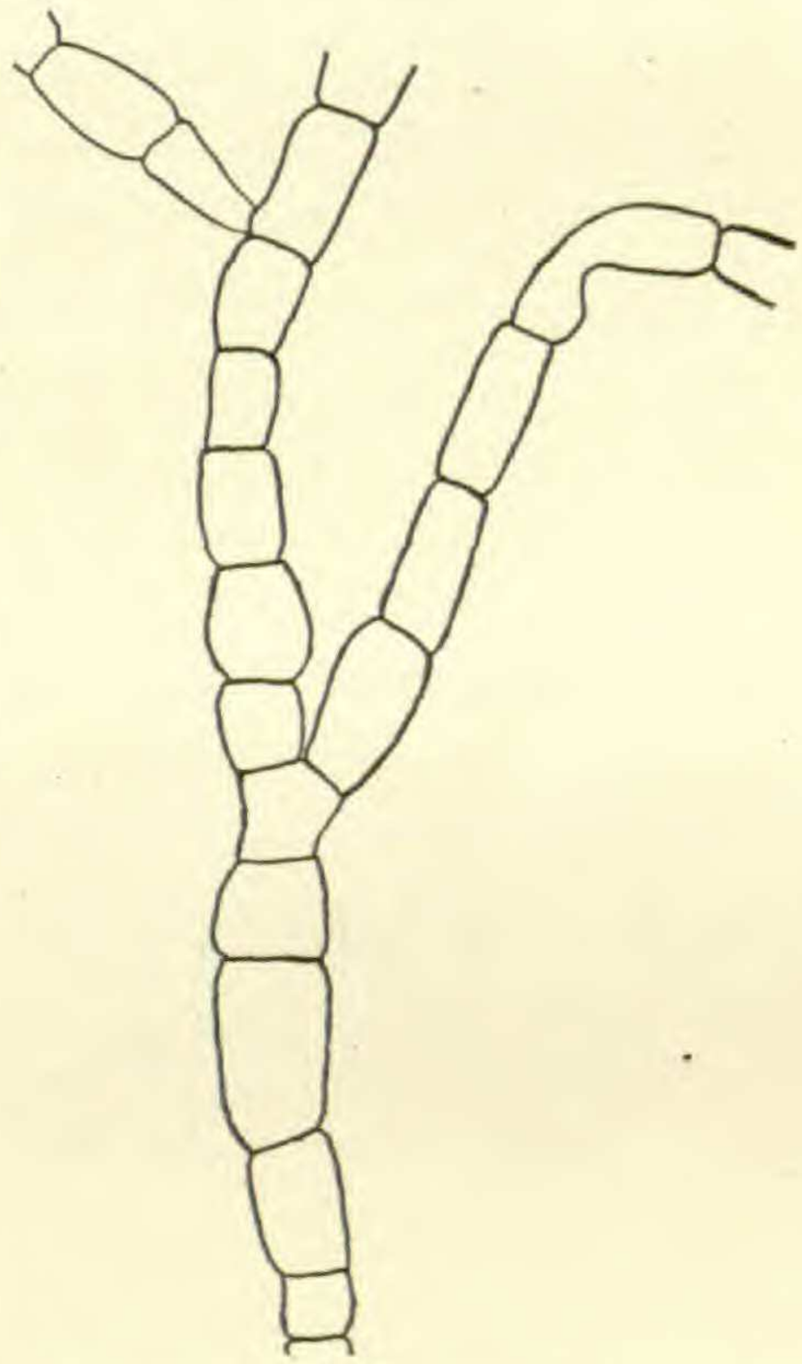

FIG. 2.—Normal hyphae, mature, showing septation with slight constriction; also the mode of branching.

ISOLATION

Diseased ray flowers were removed with sterile forceps and thrown upon solidified pea agar in Petri dishes, several to each dish. Similarly, with aseptic precautions, bits of the wood and bark from diseased peduncles, stems, and from the inside of the receptacle were plated. Each of these cultures, 25 or more in number, plated on November 18, resulted within 24 hours in pure colonies, all alike, of a fungus with a mycelium identical in appearance with that seen in diseased tissue.

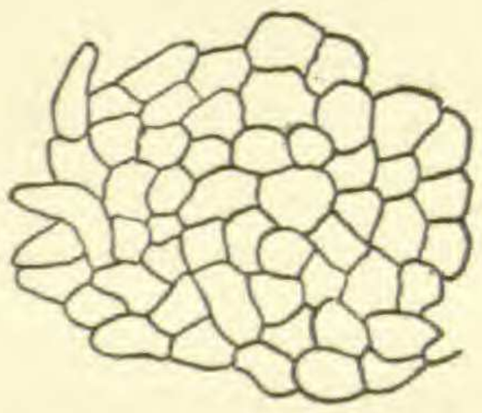

FIG. 3.—Surface view of pycnidium showing reticulation.

THE FUNGUS

The colonies grew with remarkable rapidity and remained sterile until five days old. Pycnidia were found then in the oldest portions of a colony upon one of the plates. They were amber color, as seen with the two-thirds objective and transmitted light approaching no. 30 of SAC-

CARDO's *Chromotaxia*, or the YYO⁴ of the PRANG *Standard color scale.* They were coarsely reticulated on the surface (*fig. 3*), owing to their composition of woven, coarse, mycelium threads. In diameter they ranged from 102 to 204 μ (the most usual size being about 150 μ). They were round in outline and elevated from the surface, hemispherical. Grown upon corn and also upon the apple agar, the pycnidia were very much darker, almost black. The ostiolum seemed entirely absent in many pycnidia which developed deep in the culture medium, but was present in all growing upon the surface of artificial media or upon natural substrata.

FIG. 4. — Spores from pycnidia grown on cow pea agar.

The spores are regular and oblong, or quite irregular-oblong, or swollen and rounded at one end and pointed at the other. Regular-

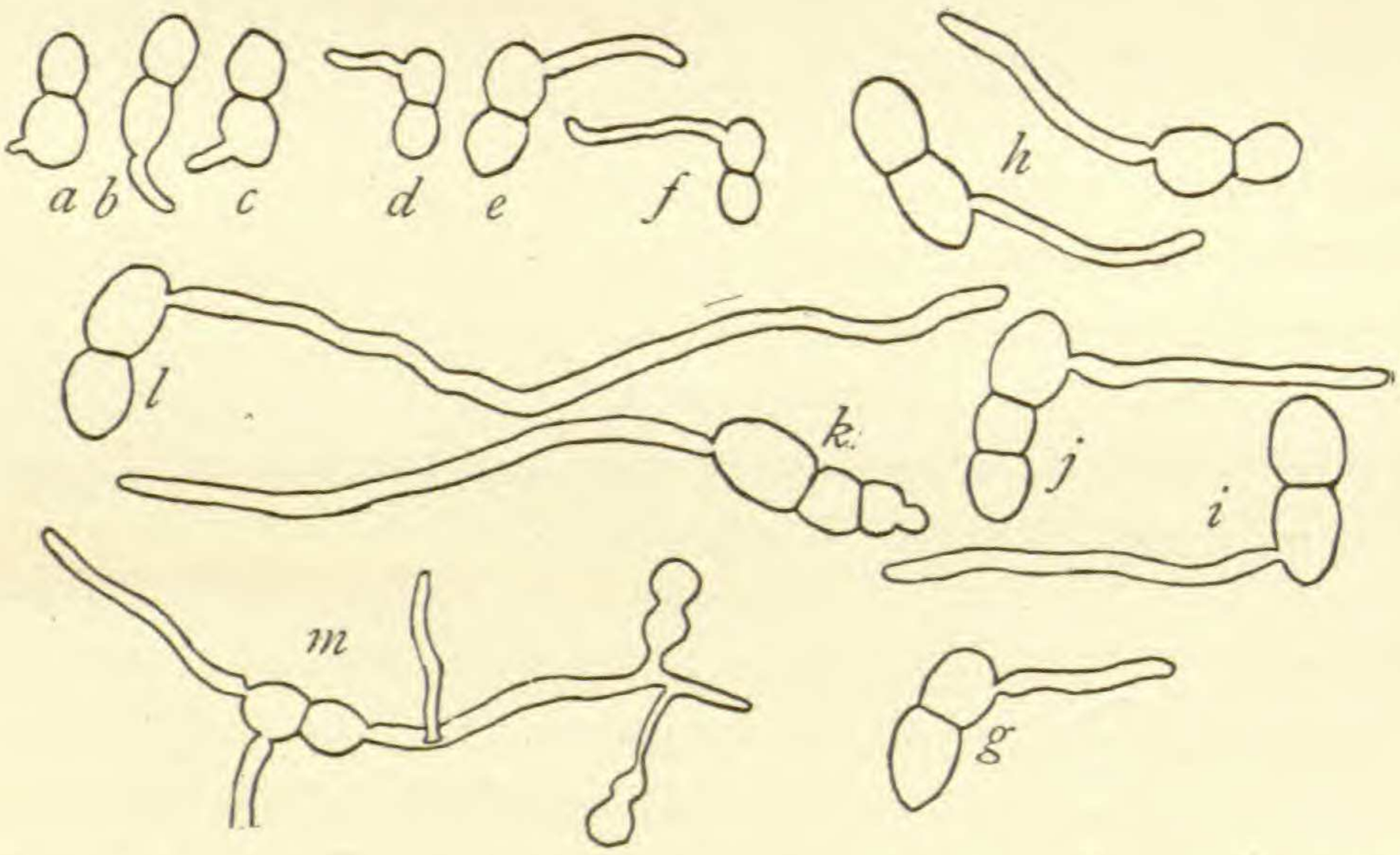

FIG. 5.—Stages in the germination of spores; *e* and *f*=*c* and *d* respectively, drawn one hour later; *c* and *d* drawn at 6 hours of age, and *e* and *f* at 7 hours; *m* 15 hours old.

oblong, however, is the prevailing type, with a pronounced tendency to irregularity (*fig. 4*). One septum, more easily seen in stained preparations, is visible in most of the older spores; though young or small spores and many larger older ones do not show it. The septum is frequently central, but there is a tendency for it to be located

nearer to one end than to the other. Rarely two septa or even three are present in one spore. The spores measure from 3 to 6.2 μ in thickness by 10 to 20 μ in length; 6.2 μ by 18 μ being the usual size. The spores are hyaline or very slightly greenish. In mass, as extruded from ripe pycnidia, they appear slightly pink. The walls are evidently mucilaginous, since the spores, as they are pushed from the pycnidia upon the absorption of water, issue in worm-like coils, cohering thus for considerable time, though they are not in visible contact. The spores germinated rapidly in water (*fig.* 5), practically every spore germinating. In germination the septum appeared in all spores, and one to three germ tubes protruded from each cell. These tubes rapidly became richly septate, and the protoplasm highly vacuolate.

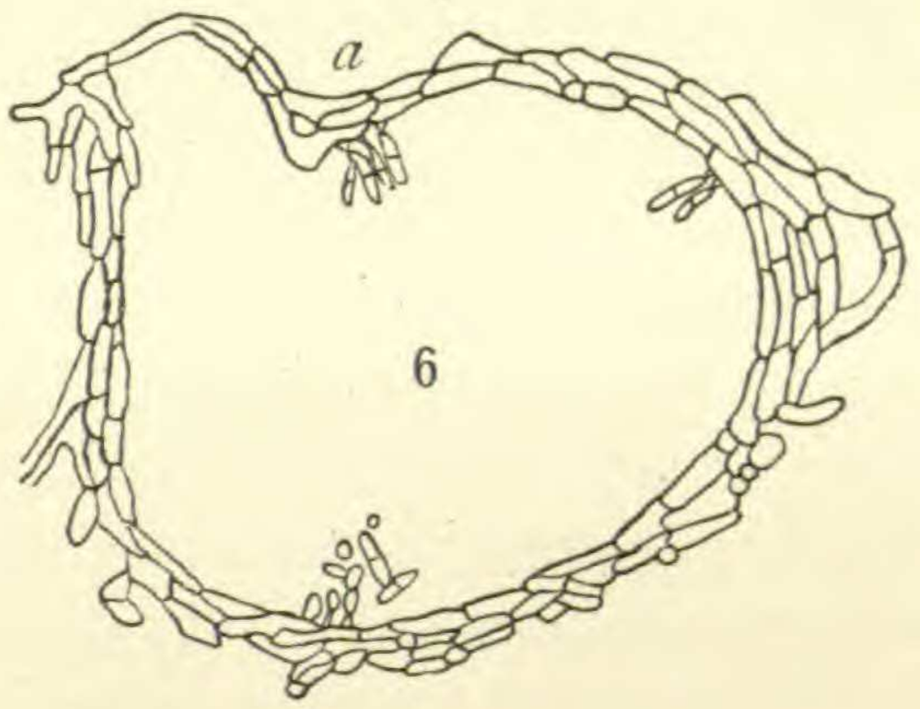

FIG. 6. Section of pycnidium. *a*, neck of ostiolum.—FIG. 7. Portion of pycnidial wall in section showing method of spore attachment.

In sections (*fig.* 6) the spores are seen to be borne on all inner portions of the pycnidial wall except the very short neck of the ostiolum, each spore originating singly (*fig.* 7) on the end of a very short stalk (about 2 to 5 μ long). These sporophores are lateral branches of the mycelial threads which constitute the pycnidial wall. The pycnidium originates within the host tissue, i. e., immersed, but rapidly enlarges to burst the overlying epidermis, so that the mature or even half-grown pycnidium appears to be superficial. There is no special aggregation of mycelium to be found below the base of the pycnidium.

The very young pycnidium is solid. Later a spore cavity develops, though apparently no more rapidly than is necessitated by spore production. In the youngest pycnidia having a spore cavity, the sur-

rounding wall is composed of several (five to eight) layers of mycelium. As the spore cavity becomes distended the wall becomes thinner, until at maturity it is composed of only one, two, or three layers of mycelium, while the region of the ostiolum becomes one layer thick and eventually ruptures.

This fungus clearly belongs to the section HYALODIDYMAE Sacc. of the SPHAERIOIDEAE Sacc. and seems closest in affinity to the members of the genus *Ascochyta*. The possession of a slight beak, which however is always very small and often entirely absent, might throw it into the genus *Rhyncophoma* Karst., but members of that genus are typically upon wood, usually saprophytic, and have a more pronounced beak.

Since no Ascochyta, or indeed any other species of the Sphaerioideae Hyalodidymae, which could by any possibility be identical with the species considered in this paper, has come to my notice as growing upon the chrysanthemum or any of its kin, I regard this as a new species, for which I give the following description.

Ascochyta Chrysanthemi, n. sp.—Perithecia few, immersed, early erumpent, single or scattered, round, hemispherical, amber colored, 100–200 μ, mostly about 150 μ; ostiolum central, small, dark bordered, often raised by a short neck; surface reticulate, pycnidia on agar media irregular, often with two ostioles and varying much in size, black in color. Mycelium abundant, innate, also superficial, aerial, floccose, richly septate.

Spores oblong, straight or irregular, 3 to 6.2 × 10 to 20 μ, mostly 6.2 × 10 μ, ends obtuse or acute; septum usually one, often obscure, rarely 2 or 3, usually without constriction until germination; protoplasm vacuolate, hyaline or light pink in mass.

HABITAT: in corollas, heads, petioles, and stems of cultivated plants of *Chrysanthemum indicum*, causing blight. North Carolina.

CULTURE CHARACTERISTICS

Thermal relation.—Cultures in Petri dishes on cow pea agar were placed in darkness at three temperatures with the results appearing in the adjoining table.

The growth both outdoors and in the incubator, was exceedingly irregular, stunted, or slow, while that of room temperature was very much more rapid.

In addition to being an unfavorable temperature for growth, the heat of the incubator produced great irregularityin the zonal development; threads started vigorously, stopped suddenly, and growth began in another direction, so that all regularity of zone formation was lost. Much of the mycelium also became incased in a dark coat, as described under the effect of cow pea leaf agar below. The plates kept at outdoor temperature, while they maintained a fair regularity in the formation of zones, did not make the same number of zones as the cultures kept at room temperature, the number of zones made in the room temperature being six while that outdoors was four.

Acid relation.—Cultures in lettuce agar in the dark at room temperature in various degrees of acidity gave the record shown on Table II on the following page:

TABLE I

INOCULATED DEC. 8, 1906. GROWTH IN MM.

December	9	10	11	12	13	14	15	16	17	18	19	Daily average
In room												
Temp. max./min.	−/−	−/−	12/20	12/20	18/21	17/21	18/20	−/−	12/18	−/−	11/17	16.19
Plate 1.....	2	4	—	12	15	18	22	25	26	33	39	3·5
Plate 2.....	2	3	—	12	15	18	22	25	26	33	39	
In incubator												
Temp. 30–35°												
Plate 35.....	3	3			contam	inated						.2.2
Plate 36.....	3	3		?	?		8	11	15	22	25	
Outdoors												
Temp. max./min.	3/17	10/18	1/14	3/7	1/16	6/22	9/22	10/21	22	14/14	−/−	−/−
Plate 37.....	0	2		?			7	11	14	19	22	2.0
Plate 38.....	0	2					7	11	14	19	22	

TABLE II

INOCULATED DECEMBER 13. GROWTH IN MM.

Plate	Drops of normal acid or alkali per tube	Fuller's scale	14	15	16	17	18	19	Average per day
25....	acid 10	+40	?	0	0				
27....	acid 5	+20	?	0	0				
49....	0	0	2 growth	3	7	12	15	18	3.1
31....	alkali 5	−19		3	5	11	14	16	2.6
29....	alkali 10	−39	?	2	3	8	9	10	1.6
34....	alkali 15	−59	?	0	0	2	3	4	.7
33....	alkali 20	−99	?	0	0	gr.	5	6	1.

It is clearly seen from these data that a neutral medium or one only slightly alkaline favors the growth of this fungus.

In the plates with 10, 15, and 20 drops of normal alkali there was no formation of zones at all. In the plate bearing 5 drops of alkali two zones were formed.

The four cultures inoculated December 8, grown at room temperature, two in light and two in darkness, agreed almost absolutely in rate of growth, a total difference of 1 or 2^{mm} noted at the end of ten days' growth being so small as to be insignificant.

Growth in various media.—Cultures in agar with various nutrients at room temperature in the dark grew as follows:

TABLE III

INOCULATED DECEMBER 13, 1906. GROWTH IN MM.

Plate	Medium	Dec. 14	Dec. 15	Dec. 16	Dec. 17	Dec. 18	Dec. 20	Dec. 21	Jan. 12
		17–21°	18–20°		12–18°				
9...	Pure agar	Grew	4	6	11				65
10..		"	4	7	11	14			70
7...	Pea agar	"	4	6	10	13			18
8...		"	4	6	10	14			18
15..	1% peptone	"	3	5	9	9			70
16..	agar	"	4	7	10	11			70
11..	1% glucose	"	5	9	15	19			80
12..	agar	"	5	8	13	17			80
13..	5% glucose	"	6	11	16	21			70
14..	agar	"	6	11	16	21			75
17..	1% starch	"	4	6	10	11			
18..	agar	"	4	7	11	12			

Plate	Medium	Dec. 8	Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec. 13	Dec. 14	Dec. 15	Jan. 12
					12–20°	12–20°	18–21°	17–21°	18–20°	
5...	Standard	Grew	1.5	2	5	9	10	11	14	52
6...	beef agar	"	1.5	2	5	10	12	13	15	32
3...	Cow pea	"	2	3	5	12	14	18	22	
4...	agar	"	2	3	5	12	14	18	22	
39..	Standard	"	2	2	4	6	8	11	15	35
40..	beef gelatin	"	2	2	4	6	10	12	15	..

Colonies grown in *pure agar* in dark or light and those upon pea agar were of the same general appearance, thin, colorless, and structureless, zone formation being entirely absent. A very few pycnidia were produced. By increasing the richness of the pea agar to 4 per cent., pycnidia were secured, but without change in other characters. The growth in pea agar was no more rapid than that on pure agar, but was much more dense; the mycelium was colorless and very few pycnidia developed.

One per cent. *peptone* adds nothing to the nutrient value of the medium for this fungus. Growth comes to an end earlier than in pure agar or other media; there are no pycnidia, no color to the mycelium, and only a very slight indication of zonation.

Glucose, 1 and 5 per cent., favor growth, the five per cent. being slightly better than the one per cent. No pycnidia developed, but there is marked, though irregular, zonation in both darkness and light, and the whole mycelium develops a striking, intense blackness. The septation and cell contents are normal and the color rests with the cell wall.

Starch, 1 per cent., did not increase the rate of growth, although upon microscopic examination it was clear that the starch grains were digested almost as soon as the advancing threads of the fungus reached them, and growth continued long in these plates. The effect of starch upon the general appearance of the colony and upon the mycelium was precisely as in the glucose plates.

In *standard beef agar* and *gelatin*, growth was about as with peptone agar, though it continued for a longer time. The mycelium was of abnormal, irregular character (*fig. 8*), and there were no pycnidia nor any development of color nor of well-marked zones.. On *beef*

gelatin the growth was more uniform than on beef agar, and the zones were somewhat clearly marked, liquefaction following in the wake of the mycelium, lagging about 4 or 5^{mm} behind the mycelial tips.

In *litmus lactose agar* the colonies developed as on beef agar without acidity.

Upon *cow pea leaf agar* the growth, while exactly parallel in rapidity with the growth upon the cow pea agar, exhibited remarkable differences in the structure of the colony, the number of zones being much less and the breadth consequently much greater. The mycelium also clung

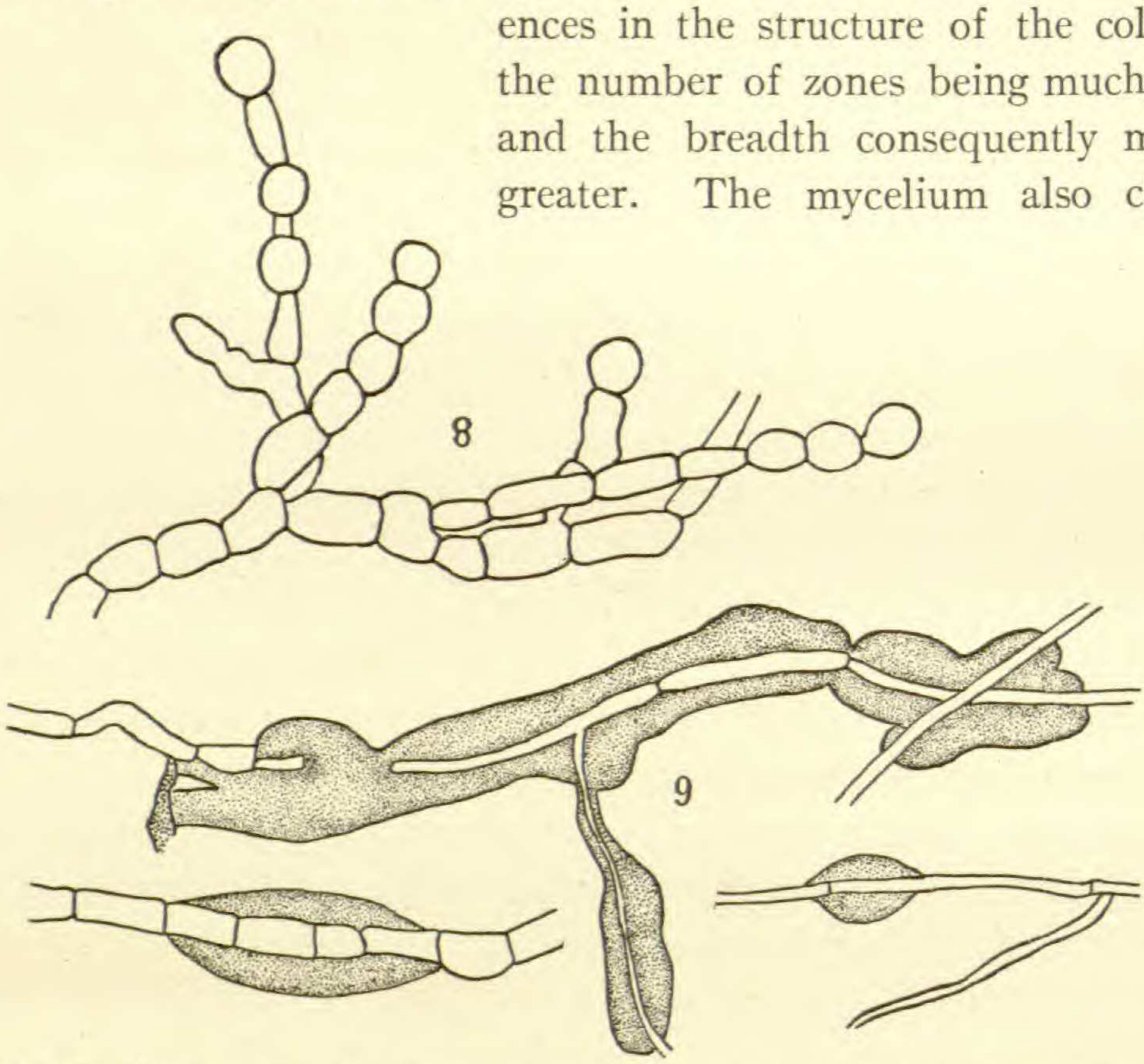

FIG. 8. Abnormal hyphae, mature, as grown in gelatin, the cells being unusually swollen.—FIG. 9. Thickening of hyphae, produced by too high temperature or an excess of nitrogenous food.

closer to the medium, was less floccose and less aerial, and in the older region of the mycelium the cells became extraordinarily thick and short and assumed a dark color. In some instances the protoplasm within the cell rounded off and became invested by a thick brown coat. In other instances a similar brown coat of much greater thickness, often four or five times as thick as the diameter of the mycelium, would form around the mycelial threads (*fig. 9*). Cow pea agar proved the best medium for the growth of this fungus. The

concentric zones formed with great regularity, five dense zones and six lesser zones being formed in a period of eleven days. In the older central portion pycnidia began to form at the end of about eight days, though often later. Pycnidial formation proceeds from the central zones to the younger zones, the pycnidia being most numerous in the denser zones, although they are by no means lacking in the lesser zones.

Upon *boiled rice*, in test tubes, the fungus grew luxuriantly. As the threads first invaded the rice there was developed a salmon color, which later turned to black.

Upon *boiled corn*, in test tubes, the mycelium developed well, became black, and pycnidia were present in abundance at the end of eleven days.

The most striking features noted in these nutrition studies were the influence of starch and glucose in agar or the starch of the rice or corn media in causing the development of an intense black coloration in the mycelium; and the unfavorable influence of peptone, beef, and gelatin as evidenced by distorted mycelium and the failure to develop pycnidia.

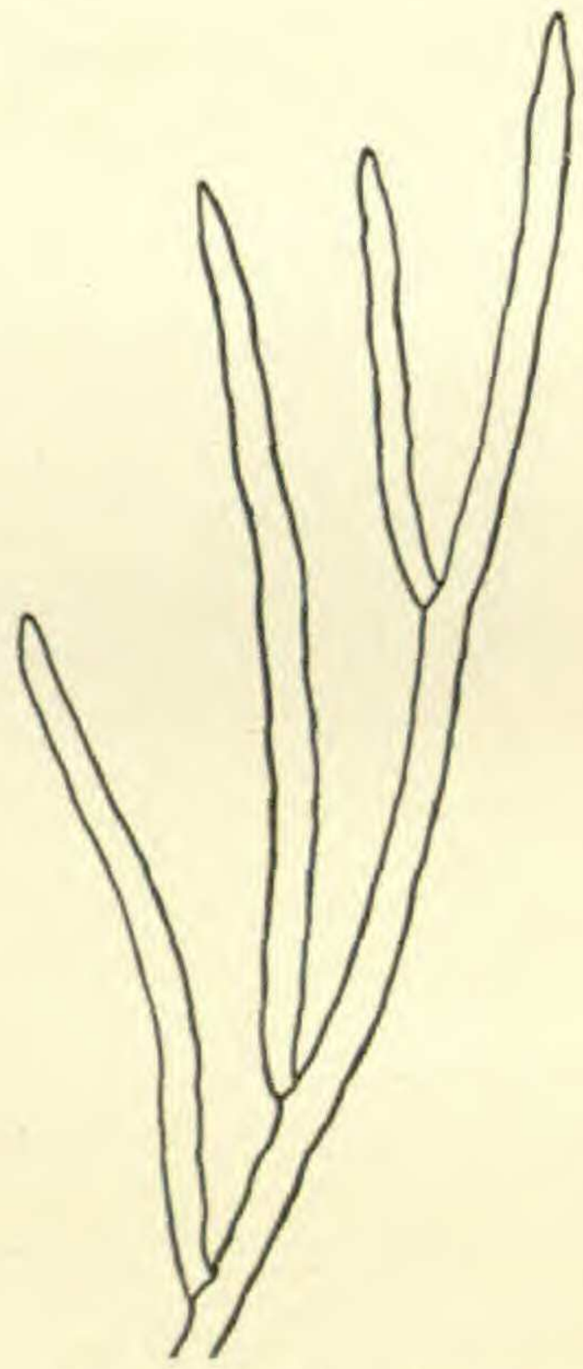

FIG. 10.—Tips of young rapidly growing hyphae.

The *formation of zones* is the most characteristic feature of growth of this fungus upon clear, solid media (*fig. 11*). Reference has constantly been made to this feature. The lesser zone, the zone which immediately surrounds the point of inoculation, is due to the mycelium growing out rapidly in every direction from the point of inoculation. As each individual thread becomes more and more distant from the center and has made more and more branches, the threads begin to crowd each other much more closely until, if conditions be proper, a time is reached when apparently the crowding becomes so great that further growth in length is inhibited. There then occurs a development of aerial hyphae and a thickening and darkening of the

ends of hyphae already produced; this results in a darker zone surrounding the lighter zone. After maintaining this condition for some time, individual threads, in a scattered fashion, break through the outer dense zone, continue their growth, and proceed to the formation of a second lesser zone, followed in course of time by a second denser zone, all being formed in precisely the same fashion that the first two zones were made. Plates have been exposed under different light relations and different temperatures, and it seems probable that

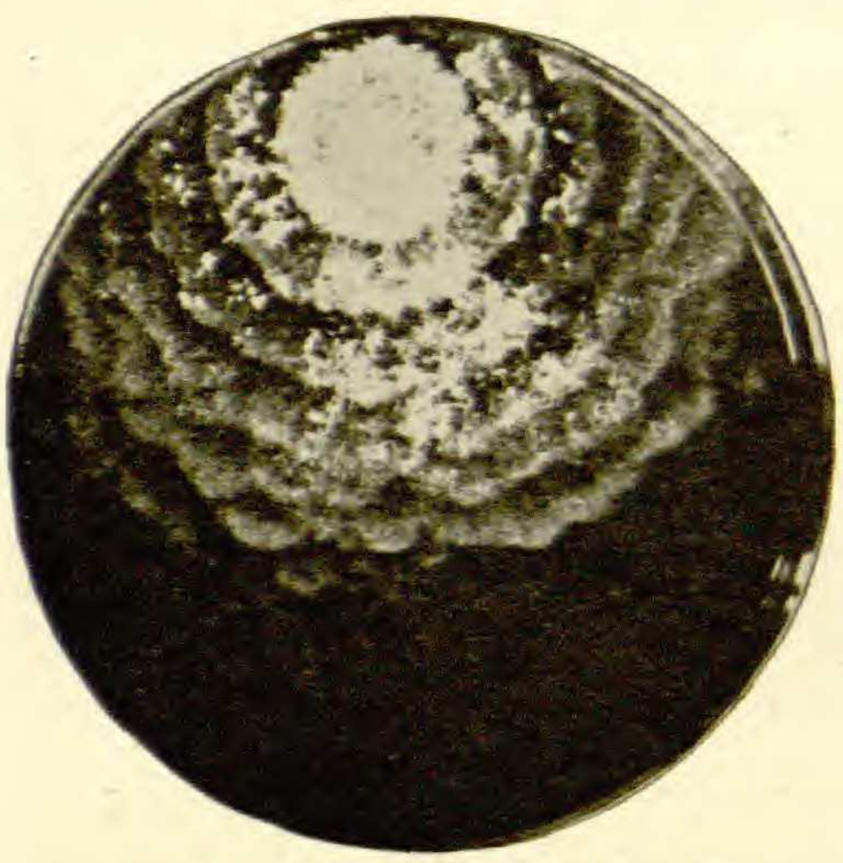

FIG. 11

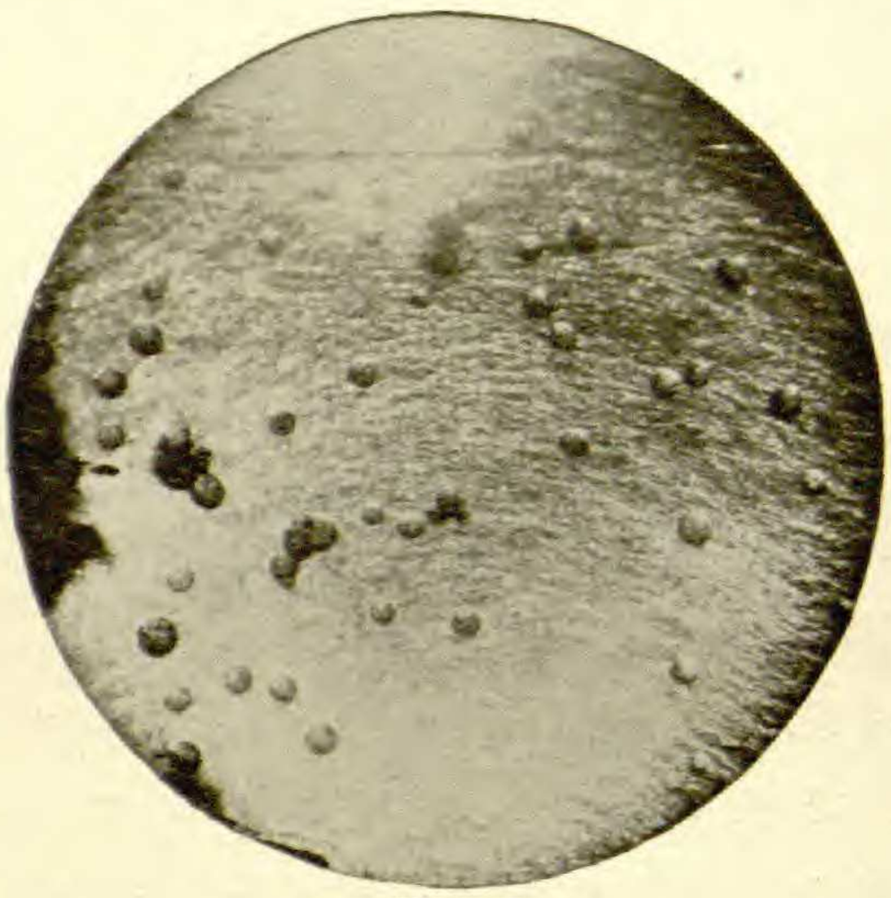

FIG. 12

FIG. 11. Pure culture of the fungus 17 days old, grown upon cow pea agar in diffuse light; pycnidia not then formed.—FIG. 12. Pycnidia on agar plate, viewed from below.

there is no connection between either of these factors and formation of zones in this fungus, other than is occasioned by increasing or decreasing growth.

INOCULATIONS (*figs. 13, 14*)

Some forty inoculations were made with mycelium taken from pure cultures on bits of agar. Of these twenty-eight gave positive results. Control cultures remained uninfected. Inoculations were made within the involucral scales, between the flowers in the head, on stems, and on leaves, as follows:

Series I

Inoculated from mycelium obtained from the original agar plate culture on which diseased rays had been placed. Inoculations made November 22, 1906.

Inoculation no. 1: under the involucre of the bud which was just beginning to open; December 8, growth observed; December 18, exhibited typical disease.

Inoculation no. 2: in the axil of the uppermost bract, in needle pricks, with agar; the bud above was just beginning to open; examined December 8 and 18 with no indication of disease.

Inoculation no. 3: with mycelium upon agar upon the end of the unopened bud; examined December 8, no growth; December 18, typical disease.

FIG. 13.—Four diseased heads produced by inoculation from pure culture.

Inoculation no. 4: control cultures similar to no. 3 with sterile needle; examined December 8 and 18, no growth.

Inoculation no. 5: at base of leaf; the tissue unbroken; examined December 8 and 18, no result.

Inoculations nos. 6, 8: agar laid upon the outside of the involucre; tissue uninjured; examined December 8 and 18, no results.

Inoculation no. 7: lost through accident.

Inoculation no. 9: at the base of the petiole; tissue uninjured; examined December 8 and 18, no result.

Inoculation no. 10: leaf base pricked and inoculated with agar culture; examined December 8 and 18, no results.

It is to be noted that in this series nos. 1 and 3 produced typical disease; that is, all of the inoculations which were attempted within

the involucre. Control inoculations produced no growth, and attempts to produce the disease upon leaves and stems through uninjured tissue failed.

Series II

Inoculated November 24, 1906, with mycelium which was richly set with pycnidia.

Inoculations nos. 11, 12: agar bearing mycelium placed deep between the petals of the open flower; examined December 8, showed typical disease.

Inoculation no. 13: same as no. 11 using sterile agar; control inoculation; examined December 8 and 18, no growth.

Inoculation no. 14: peduncle immediately below the bud scarified and inoculated with mycelium on agar; examined December 8, typical disease.

Inoculation no. 15: similar to no. 14, but with sterile agar; control experiment; examined December 8 and 18, no growth.

Inoculation no. 16: upon scarified stem immediately under head with agar bearing mycelium, the whole covered with wet cotton; examined December 8 and 18, no result.

Inoculation no. 17: conditions same as in no. 16; examined December 18, head typically diseased.

Inoculation no. 18: conditions same as in no. 16; examined December 8, no result; December 10, typical disease.

Inoculation no. 19: agar bearing mycelium placed upon the tip of the opening head; examined December 8, no growth; December 18, typical disease.

Inoculation no. 20: conditions same as in no. 19; examined December 8, typical disease.

Inoculation no. 21: conditions same as in no. 19; lost through accident.

It is to be observed that in this series the inoculations within the flower produced typical disease. Control inoculations produced no disease. Inoculation upon scarified stem when covered with damp cotton to prevent evaporation was capable of inducing the disease.

FIG. 14

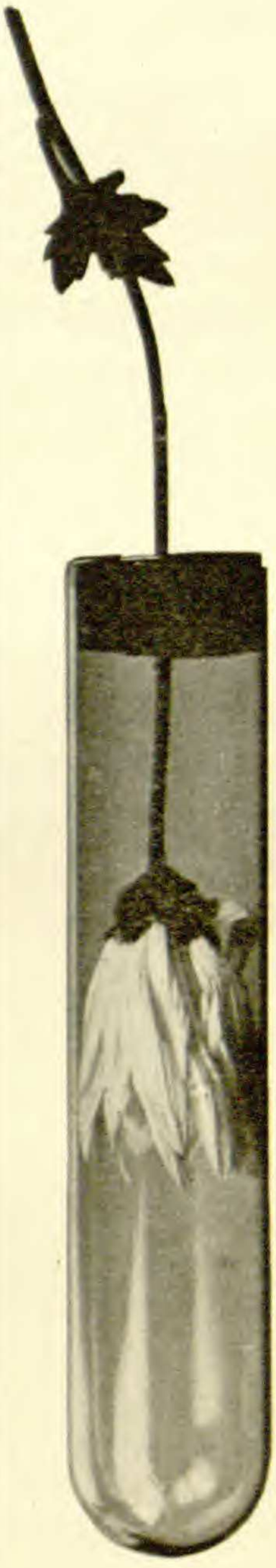

FIG. 15

FIG. 14. Plant bearing flowers in condition of disease; produced by inoculation. *1*, The topmost head inoculated between the flowers with pycnidia from agar. *2*, The second tallest head as in no. 1. *4*, Inoculated on scarified bark immediately below the head. Dates as above. *13*, Inoculated no. 29 inside involucre, covered by tube 24 hours; diseased December 8. *8*, Inoculated November 24 with agar on scarified bark under wet cotton; diseased December 10.—FIG. 15. Showing method of covering inoculated heads to supply humid atmosphere.

Series III

Inoculated November 29, 1906.

Inoculation no. 22: agar bearing mycelium was placed between the rays of a head and the whole covered with a tube and supplied with wet cotton to prevent evaporation; tube and cotton removed next day; examined December 8, typical disease.

Inoculation no. 23: similar to no. 22 but inoculation made just inside the involucre; examined December 8, typical disease.

Inoculation no. 24: similar to no. 22 but upon the tip of the bud; covering tube removed after two days; examined December 8, typical disease.

Inoculation no. 25: a single ray from a diseased flower placed among the rays of a flower just beginning to open, which were left uncovered; examined December 8, typical disease.

Inoculation no. 26: similar to no. 25; but covered with tube as in no. 22; examined December 8, typical disease.

Inoculation no. 27: similar to no. 22; examined December 8, typical disease.

From inoculations nos. 22, 23, 24, and 27 it seems evident the protection of the inoculations from evaporation for 24 or 48 hours leads to greater certainty and rapidity of infection. Inoculations nos. 25 and 26 go to show the lack of necessity for any covering when diseased rays are used with which to inoculate. It is possible that the mycelium is in a more vigorous condition in such diseased rays.

Series IV

Inoculations made November 30, 1906, from very vigorously growing mycelium from a plate of cow pea agar.

Inoculations 28 to 31: made from the extreme tip of the mycelium taken from the edge of the colony; inoculation under the outer rays; examined December 8, no growth; December 18, typical disease in all.

Inoculations 32 to 35: inoculating material taken from the youngest dense zone in the colony; inoculated as in nos. 28 to 31; examined December 8, no growth; December 18, all typically diseased.

Inoculations 36 to 40: inoculating material taken from the center

of the colony, the agar being full of old, dark, thick hyphae; examined December 8, all showed typical cases of disease.

It seems apparent from this series that the mycelium from the older central portions is capable of producing quicker infection than that from the outer region of the colony.

RECOVERY OF FUNGUS FROM INOCULATED HEADS

In many cases rays were taken from the heads which were made diseased by inoculation and these rays were thrown upon Petri dishes containing solidified pea agar, with the result that in each case the petal was soon the center of the pure colony of a fungus identical with that used in the inoculation.

As a result of all of these inoculations it seems clear that the fungus in question is actually the cause of the diseased condition of the blossom; that it can gain entrance to the tissue of the blossom much more easily than to the tissue of the leaves, petioles, and stems; and that humidity at the time of inoculation favors the success of the inoculation.

I desire to acknowledge my indebtedness to Mr. J. G. Hall, Assistant Pathologist, for the illustrations accompanying this article.

MEDIA USED

Cow pea agar: 500 grams of fresh cow pea pods and contents were stewed in one liter of water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

Cow pea leaf agar: 500 grams of cow pea leaves and petioles were stewed in one liter of water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

Pea agar: ten garden peas were autoclaved with 1 liter of water for one-half hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

Four per cent. pea agar: four per cent. by weight of dry peas were stewed with 1 liter of water for one-half hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

Standard beef agar: according to the rules of the American Public Health Association.

Standard beef gelatin: according to the rules of the American Public Health Association.

Pure agar: 1 per cent. agar without addition of nutrients.

Litmus lactose agar: standard beef agar with the addition of 1 per cent. lactose, and litmus sufficient to color.

Lettuce agar: 10 grams of lettuce leaves and petioles were stewed in water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

Boiled rice: 2 grams of washed rice to 8^{cc} of water, tubed, autoclaved at 110°.

Boiled corn: 2 grams of washed corn to 8^{cc} of water, tubed, autoclaved at 110°.

Glucose agar and *starch agar:* made by adding percentages indicated to pure agar, and sterilizing intermittently.

Peptone agar: made by adding per cent. indicated to pure agar and autoclaving at 110°.

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